

Patent Claims

1. Nucleic acid molecule, comprising a nucleic acid coding for a polypeptide with chorismate mutase activity or complementary strand thereof, wherein the nucleic acid is selected from
- (a) a nucleic acid with the DNA sequence stated in SEQ ID NO: 1 or the RNA sequence corresponding thereto;
  - (b) a nucleic acid which hybridises with the complementary strand of a nucleic acid according to (a);
  - (c) a nucleic acid which on the basis of the genetic code is degenerate to the DNA sequences defined in (a) and (b);
  - (d) a nucleic acid which hybridises with one of the nucleic acids stated in (a) to (c) and the complementary strand whereof codes for a polypeptide with chorismate mutase activity;
  - (e) a nucleic acid which is at least 60% homologous to the nucleic acid stated in (a);
  - (f) a variant of the nucleic acids stated in (a) to (e), wherein the variant has additions, deletions, insertions or inversions relative to the nucleic acids stated in (a) to (e);
  - (g) a fragment of one of the nucleic acids stated in (a) to (f); *117, 2*
  - (h) a combination of several of the nucleic acids stated in (a) to (g),

wherein the polypeptide encoded by the nucleic acid or complementary strand thereof has at least 10% of the chorismate mutase activity of the chorismate mutase according to SEQ ID NO:2, with the proviso that the nucleic acid molecule does not include the nucleic acid sequence of the ARO7 gene from *Saccharomyces cerevisiae*.

2. Nucleic acid molecule according to Claim 1, **characterised in that** it is a desoxyribo-nucleic acid molecule.
3. Nucleic acid molecule according to Claim 1, **characterised in that** the hybridisation stated under (b) or (d) is performed under stringent conditions.
4. Nucleic acid molecule according to Claim 1, **characterised in that** the nucleic acid stated under (e) is at least 80% homologous to one of the nucleic acids stated under (a).
5. Nucleic acid molecule according to Claim 1, **characterised in that** the nucleic acid stated under (e) is at least 90% homologous to one of the nucleic acids stated under (a).
6. Nucleic acid molecule according to Claim 1, **characterised in that** the nucleic acid stated under (e) is at least 95% homologous to one of the nucleic acids stated under (a).
7. Nucleic acid molecule according to Claim 1, **characterised in that** the polypeptide encoded by the nucleic acid has at least 50% of the chorismate mutase activity of the chorismate mutase according to SEQ ID NO:2.
8. Nucleic acid molecule according to Claim 1, **characterised in that** the polypeptide encoded by the nucleic acid has at least 75% of the chorismate mutase activity of the chorismate mutase according to SEQ ID NO:2.
9. Nucleic acid molecule according to Claim 1, further comprising a promoter suitable for expression control, wherein the nucleic acid coding for a polypeptide with chorismate mutase activity is under the control of the promoter.
10. Nucleic acid molecule according to Claim 9, **characterised in that** the promoter is the MOX promoter or the FMD promoter from *Hansenula polymorpha*.
11. Nucleic acid molecule according to Claim 9, further comprising a heterologous nucleic acid sequence suitable for expression and optionally secretion.

12. Nucleic acid molecule according to Claim 9, wherein the nucleic acid molecule contains at least a part of a vector, wherein the vector is selected from: bacteriophages, plasmids, adenoviruses, vaccinia viruses, baculoviruses, SV4O virus and retroviruses.

13. Nucleic acid molecule according to Claim 9, wherein the nucleic acid further comprises a His-tag coding nucleic acid sequence and the expression of the nucleic acid molecule leads to the formation of a fusion protein with a His-tag.

14. Non-naturally occurring host cell, containing a nucleic acid molecule according to Claim 9, wherein the host cell is a prokaryotic or eukaryotic cell suitable for the expression of the nucleic acid molecule.

15. Host cell according to Claim 14, **characterised in that** the prokaryotic cell is selected from *E. coli* and *Bacillus subtilis*.

16. Non-naturally occurring host cell according to Claim 14, **characterised in that** the eukaryotic cell is selected from yeast cells such as *Hansenula polymorpha* and *Saccharomyces cerevisiae*, insect cells and mammalian cells, preferably from CHO cells, COS cells and HeLa cells.

17. Process for the production of a polypeptide with chorismate mutase activity, wherein the nucleic acid molecule according to Claim 1 is expressed in a suitable host cell and the protein is isolated if necessary.

18. Process according to Claim 17, **characterised in that** the polypeptide with chorismate mutase activity produced is naturally or chemically modified.

19. Process according to Claim 17, **characterised in that** the expression is performed in a host cell according to Claim 14.

20. Process for the production of a phenylalanine- and tyrosine-auxotrophic yeast strain, comprising the destruction of the endogenous chorismate mutase gene of the corresponding yeast strain, wherein the mutant has less than 10% of the chorismate mutase activity of the chorismate mutase according to SEQ ID No:2.

21. Process according to Claim 20, comprising the following steps:

- a) Preparation of a construct, comprising at least two fragments of a nucleic acid according to Claim 1 (a) to 1 (h) suitable for homologous recombination; which flank a nucleic acid not suitable for homologous recombination;
- b) Transformation of cells of a yeast strain with intact endogenous chorismate mutase gene with this construct and
- c) Identification of phenylalanine- and tyrosine-auxotrophic transformants.

22. Process according to Claim 21, **characterised in that** the process further comprises the selection of the transformants on phenylalanine/tyrosine-deficient medium.

23. Process according to Claim 20, **characterised in that** the construct also comprises a selection marker gene.

24. Process according to Claim 21, **characterised in that** the construct further comprises one or several recombination sites.

25. Process according to Claim 24, **characterised in that** one recombination site is loxP.

26. Process according to Claim 25, **characterised in that** the cells of the yeast strain in step b) are further brought into contact with nucleic acid suitable for the expression of Cre recombinase.

27. Process according to Claim 20, **characterised in that** the yeast strain is *Hansenula polymorpha*.
28. Phenylalanine- and tyrosine-auxotrophic yeast strain, obtainable by the process according to Claim 20.
29. Auxotrophic yeast strain according to Claim 28, **characterised in that** it is a mutant of a prototrophic yeast strain of *Hansenula polymorpha*.
30. Process for the recombinant production of proteins, comprising:
- Transformation of the auxotrophic yeast strain according to Claim 28 with a combination of a nucleic acid molecule suitable for expression according Claim 9 and a heterologous gene suitable for expression under the control of a suitable promoter; and
  - Culturing of the transformants under conditions suitable for the expression of the heterologous gene and the nucleic acid molecule and optionally isolation of the protein which is encoded by the heterologous gene.
31. Process according to Claim 30, **characterised in that** the process further includes the step of the selection of the transformants on phenylalanine- and/or tyrosine-deficient medium.
32. Process according to Claim 30, characterised in that the nucleic acid molecule and the heterologous gene are separate from one another.
33. Process according to Claim 30, characterised in that the nucleic acid molecule and the heterologous gene are present in one vector.